

# COBAS AmpliScreen™ HCV Test, v2.0

## Package Insert

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# COBAS AmpliScreen™ HCV Test, v2.0 (96 Test)

COBAS AmpliScreen HCV Test, version 2.0	96 Tests	P/N: 3302563018
COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit	96 Tests	P/N: 3302555018
COBAS AMPLICOR™ Wash Buffer	500 Tests	P/N: 20759899123 ART: 07 5989 9 US: 83314

## Intended Use

The COBAS AmpliScreen Hepatitis C Virus (HCV) Test, version 2.0 (v2.0) is a qualitative *in vitro* test for the direct detection of Hepatitis C Virus RNA in human plasma from donations of whole blood and blood components for transfusion.

The test is intended for use in screening individual donor samples of human plasma, or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The test is intended to be used for detecting HCV RNA in conjunction with licensed tests for detecting antibodies to HCV.

This assay is not intended for use as an aid in diagnosis.

## Summary and Explanation of the Test

Hepatitis C Virus is considered to be the principal etiologic agent responsible for 90-95% of the cases of post-transfusion non-A and non-B hepatitis.<sup>1,2</sup> HCV is a single-stranded, positive sense RNA virus with a genome of approximately 10,000 nucleotides coding for 3,000 amino acids.<sup>1</sup> As a blood-borne virus, HCV can be transmitted by blood and blood products. The global prevalence of HCV infection, as determined by immunoserology, ranges from 0.6% in Canada to 1.5% in Japan.<sup>2</sup>

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products.<sup>3-6</sup> Recent studies indicate that nucleic acid-based amplification tests for HCV RNA will allow detection of HCV infection earlier than the current antibody based tests. Nucleic acid testing (NAT) of whole blood donations has been in place in the United States since 1999 under Investigational New Drug Application (IND). Nucleic acid-based tests can detect viremic units donated by carriers who do not seroconvert or who lack antibodies to serological markers normally detected by immunological assays.<sup>7-9</sup>

The COBAS AmpliScreen HCV Test, v2.0, uses a generic sample preparation technique in a mini-pool testing format along with automated amplification and detection using PCR on the COBAS AMPLICOR™ Analyzer for the detection of HCV RNA in blood donations. The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as AmpErase® to reduce potential contamination by previously amplified material (amplicon).

## Principles of the Procedure

The COBAS AmpliScreen HCV Test, v2.0 is based on five major processes:

1. Sample Processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA)<sup>10</sup>
3. PCR amplification<sup>10</sup> of target cDNA using HCV-specific complementary primers
4. Hybridization of the amplified products to oligonucleotide probes specific to the target(s)
5. Detection of the probe-bound amplified products by colorimetric determination.

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## Sample Processing

Two specimen processing procedures are used with the AmpliScreen HCV Test, v2.0 as follows:

- Multiprep Specimen Processing Procedure for preparation of mini-pool specimens
- Standard Sample Processing for preparation of individual donor samples

In the Standard Specimen Processing Procedure, HCV RNA is isolated directly from plasma by lysis of the virus particles with Multiprep Lysis Reagent followed by precipitation of the RNA with alcohol. In the Multiprep Specimen Processing Procedure, HCV viral particles are first pelleted from the plasma sample by high speed centrifugation, followed by lysis of the pelleted virus with a chaotropic agent (Multiprep Lysis Reagent) and precipitation of the RNA with alcohol.

The Multiprep Internal Control (MP IC), containing the HCV Internal Control, is introduced into each sample with the Multiprep Lysis Reagent and serves as an extraction and amplification control for each processed specimen and control. The HCV Internal Control is an RNA transcript with primer binding regions identical to those of the HCV target sequence, a randomized internal sequence of similar length and base composition as the HCV target sequence, and a unique probe binding region that differentiates the HCV Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HCV Internal Control and the HCV target RNA.

## Reverse Transcription

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (*rTth* pol). In the presence of manganese ( $Mn^{2+}$ ) and under the appropriate buffer conditions, *rTth* pol has both reverse transcriptase and DNA polymerase activity.<sup>18</sup> This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse

transcription using *rTth* pol produces a cDNA copy of the HCV target and the HCV Internal Control RNA.

## PCR Amplification

Following reverse transcription using *rTth* pol, a second DNA strand is produced from the cDNA copy, thereby yielding a double-stranded DNA copy of the HCV target and HCV Internal Control RNA. The reaction mixture is heated to separate the resulting double-stranded DNA. As the mixture cools, primers anneal to the target DNA and in the presence of  $Mn^{2+}$  and excess deoxynucleotide triphosphates (dNTPs), extend the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA. The required number of cycles is preprogrammed in the COBAS AMPLICOR Analyzer.

## Selective Amplification

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, AmpErase® (uracil-N-glycosylase, UNG) is added to the COBAS AmpliScreen HCV Test, v2.0. AmpErase recognizes and catalyzes the destruction of DNA strands containing deoxyuridine<sup>11</sup>, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate in place of deoxythymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase before amplification of the target DNA. AmpErase, which is included in the Master Mix reagent, catalyzes the cleavage of DNA, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target

amplicon. Following amplification, any residual enzyme is denatured by the addition of the denaturation solution, thereby preventing the degradation of any target amplicon.

## Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds denaturation solution to the A-tubes to chemically denature the HCV amplicon and the HCV Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to two detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for HCV amplicon or HCV Internal Control amplicon is added to the individual D-cups. The biotin-labeled HCV target and HCV Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. This hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

## Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles in the D-cups to remove unbound material, and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase Conjugate binds to the hybridized biotin-labeled amplicon. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) to each D-cup. In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The absorbance is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

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## Materials Provided by Roche

The COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit and the COBAS AMPLICOR Wash Buffer kit are provided as stand-alone kits to be used in conjunction with the COBAS AmpliScreen HCV Test, v2.0, as well as the COBAS AmpliScreen HIV-1 Test, v1.5, and the COBAS AmpliScreen HBV Test.

### **COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit (96 tests) (P/N: 3302555018)**

MP (+) C	(Multiprep Positive (+) Control)
MP LYS	(Multiprep Lysis Reagent)
MP DIL	(Multiprep Specimen Diluent)
MP IC	(Multiprep Internal Control)
MP (-) C	(Multiprep Negative (-) Control)
NHP	(Negative Plasma (Human))

### **COBAS AmpliScreen HCV Test, version 2.0 (96 tests) (P/N: 3302563018)**

#### **COBAS AmpliScreen HCV Amplification Reagents, version 2.0**

HCV MMX, v2.0	(HCV Master Mix, version 2.0)
HCV Mn <sup>2+</sup> , v2.0	(HCV Manganese Solution, version 2.0)

**COBAS AmpliScreen HCV Detection Reagents, version 2.0**

DN4	(Denaturation Solution)
CH PS1, v2.0	(HCV Probe Suspension 1, version 2.0)
CH4, v2.0	(HCV Probe Suspension 2, version 2.0)
CI PS1	(IC Probe Suspension 1)
CI4	(IC Probe Suspension 2)
CN4	(Avidin - Horseradish Peroxidase Conjugate)
SB3	(Substrate A)
SB	(Substrate B)

**COBAS AMPLICOR Wash Buffer Kit (500 tests)**  
**(P/N: 20759899123; ART: 07 5989 9; US: 83314)**

WB	(10X-Wash Concentrate)
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## Other Materials Required But Sold Separately (May Be Purchased from Roche)

- COBAS AMPLICOR Analyzer with software version 0022B, Printer, and *Operator's Manual*
- COBAS AMPLICOR A-rings
- COBAS AMPLICOR D-cups
- AMPLILINK™ Software version 1.3 and *Operator's Manual*
- Hamilton MICROLAB AT plus 2 Pipettor, the COBAS AmpliScreen Pooling System Guide, Software Kit (Hamilton SUNPLUS and RUNENDE Software, and the Roche Pooling Methods Software version 1.4)
- Sarstedt 1.5-mL tube Barcode Labels
- Hamilton Archive and Intermediate Plate Barcode Labels
- Refrigerated high speed centrifuge with fixed angle rotor (45 degrees, capacity for at least 24 x 1.5-mL tubes) with an RCF of 23,600 x g (Heraeus Centrifuge 17RS or Biofuge 28RS with HFA 22.1 rotor, Heraeus Biofuge Stratos with the 3331 rotor or equivalent)

## Materials Required But Not Provided by Roche

- Microcentrifuge, (max. RCF 16,000 x g, min. RCF 12,500 x g) (Eppendorf® 5415C, HERMLE Z230M, or equivalent)
- Eppendorf 1.25 mL Combitip® Reservoir (sterile) or equivalent
- Eppendorf Multipette® pipette or equivalent
- Ethanol, 90% or 95%, reagent grade for Molecular Biology or Histology use
- Distilled or deionized water
- Powderless disposable gloves

- Isopropyl alcohol, reagent grade
- Disposable, Sterile, Polystyrene Pipettes (5 mL, 10 mL and 25 mL)
- Sterile, RNase-free, fine-tip transfer pipettes
- Pipettors (capacity 20 µL to 1000 µL, capable of providing  $\pm 3\%$  accuracy and precision  $\leq 5\%$ ) with aerosol barrier or positive displacement RNase-free tips
- Tube racks (Sarstedt P/N 93.1428 or equivalent)
- 1.5-mL sterile, non-siliconized, conical polypropylene screw-cap tubes, (Sarstedt 72.692.105 or equivalent)
- Vortex mixer
- Hamilton Slotted Deepwell Archive Plate, 2.2 mL and Sealing Capmat
- Hamilton Slotted Intermediate Plate

## Reagents

### COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, 96 Tests

**MP (+) C** (Multiprep Positive (+) Control) 8 x 0.1 mL

Tris-HCl buffered solution containing noninfectious RNA transcripts for HCV and HIV-1 and noninfectious HBV DNA plasmid with EDTA and sodium azide as a preservative.

**MP LYS** (Multiprep Lysis Reagent) 8 x 9.0 mL

Tris-HCl buffered solution with Dithiothreitol, Glycogen and Guanidine thiocyanate

Xn



Harmful

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<b>MP DIL</b>	(Multiprep Specimen Diluent)	8 x 4.8 mL
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Tris-HCl buffered solution with EDTA and 0.05% sodium azide as a preservative

<b>MP IC</b>	(Multiprep Internal Control)	8 x 0.1 mL
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Tris-HCl buffered solution with non-infectious internal control RNA transcripts for HCV and HIV-1 and DNA plasmid for HBV, Poly rA RNA, EDTA, indicator dye and 0.05% sodium azide as a preservative

<b>MP (-) C</b>	(Multiprep Negative (-) Control)	8 x 0.1 mL
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Poly rA RNA, EDTA and 0.05% sodium azide as a preservative

<b>NHP</b>	(Negative Plasma (Human))	16 x 1.6 mL
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Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg, with 0.1% ProClin® 300 as a preservative

## **COBAS AmpliScreen HCV Test, version 2.0, 96 Tests**

### **Amplification Reagents**

<b>HCV MMX, v2.0</b>	(HCV Master Mix, version 2.0)	8 x 0.7 mL
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Bicine buffered solution with DMSO, glycerol, rTth DNA Polymerase, potassium acetate, primers, dNTPs, AmpErase and 0.05% sodium azide as a preservative

<b>HCV Mn<sup>2+</sup>, v2.0</b>	(HCV Manganese Solution, version 2.0)	8 x 0.1 mL
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Manganese solution with acetic acid, indicator dye and 0.05% sodium azide as a preservative

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## COBAS AmpliScreen HCV Detection Reagents, version 2.0

**DN4** (Denaturation Solution) 1 x 100 Tests

EDTA

Thymol blue

Xi 1.6% (w/w) Sodium hydroxide



Irritant

**CH PS1 v2.0** (HCV Probe Suspension 1, version 2.0) 1 x 100 Tests

MES buffer solution containing capture oligonucleotides and magnetic microparticles with 0.05% sodium azide as a preservative

**CH4, v2.0** (HCV Probe Suspension 2, version 2.0) 1 x 100 Tests

Sodium phosphate buffer

34.7% Sodium thiocyanate

0.2% Solubilizer

Xn 34.7% (w/w) Sodium thiocyanate



Harmful

**CI PS1** (IC Probe Suspension 1) 1 x 100 Tests

MES buffer solution containing magnetic microparticles with capture oligonucleotides and 0.09% sodium azide as a preservative

**CI4** (IC Probe Suspension 2) 1 x 100 Tests

Sodium phosphate buffer containing 24.9% sodium thiocyanate

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**CN4** (Avidin-Horseradish Peroxidase Conjugate) 2 x 100 Tests

Tris-HCl buffer solution containing Avidin-horseradish peroxidase conjugate, bovine serum albumin, Emulsit 25 and phenol with ProClin 150 as a preservative

**SB3** (Substrate A) 10 x 75 Tests

Citrate solution containing hydrogen peroxide with ProClin 150 as a preservative

**SB** (Substrate B) 10 x 75 Test

0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)

40% Dimethylformamide (DMF)

T 40% (w/w) Dimethylformamide (DMF)



Toxic

R: 61-20/21-36 May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to eyes.

S: 53-45 Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**COBAS AMPLICOR Wash Buffer, 500 Tests**

**WB** (10X-Wash Concentrate) 2 x 250 Tests

Phosphate buffer solution containing detergent with ProClin 300 as a preservative

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## Storage Instructions

- A. Room Temperature is defined as 15 – 30°C.
- B. Do not freeze reagents.
- C. Store the following reagents at 2 – 8°C. Unopened, these reagents are stable until the expiration date indicated.

**MP LYS, MP IC, MP (+) C, MP (–) C, MP DIL and NHP**

**HCV MMX, v2.0 and HCV Mn<sup>2+</sup>, v2.0**

**CH PS1, v2.0, CH4, v2.0, CI PS1 and CI4**

**CN4, SB3 and SB**

- D. Store **DN4** and **WB** at 2 – 25°C. **DN4** and **WB** are stable until the expiration dates indicated.
- E. Do not expose **SB3, SB** or Working Substrate to metals, oxidizing agents or direct sunlight.
- F. The following reagents are one time use. Discard any unused portion.

**MP IC, MP (+) C, MP (–) C, MP DIL and NHP**

**HCV Mn<sup>2+</sup>, v2.0, CH4, v2.0, CI4 and SB**

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## Precautions

### For *In Vitro* Diagnostic use

- A. Specimens may be infectious. Use Universal Precautions when performing the assay.<sup>12, 13</sup> Only personnel proficient in the use of the COBAS AmpliScreen System and trained in handling infectious materials should perform this procedure. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70% ethanol.
- B. **CAUTION: The Negative Human Plasma of this kit contains human blood products non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood-sourced materials should be considered potentially infectious and should be handled with Universal Precautions.** If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.
- C. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- D. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

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- E. **Heparin has been shown to inhibit PCR. Do not use heparinized plasma with this procedure.**
- F. Use only supplied or specified required disposables to ensure optimal assay performance.
- G. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. ***Do not use snap cap tubes.***
- H. Adequately vortex, where specified, to ensure optimal assay performance.
- I. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
- J. Before use, visually inspect each reagent bottle to ensure that there are no signs of leakage and/or abnormal color. If there is any evidence of leakage and/or abnormal color, do not use that bottle for testing.
- K. Dispose of all materials that have come in contact with specimens and reagents in accordance with country, federal, state and local regulations.
- L. Do not use a kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers. Do not use expired reagents.
- M. Material Safety Data Sheets (MSDS) are available on request.
- N. Supplies and equipment must be dedicated to each pre-amplification activity and should not be used for other activities or move between areas. ***Fresh, clean gloves must be worn in each area and must be changed before leaving that area.***  
Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.
- O. Avoid contact of **MP LYS, HCV MMX, v2.0, HCV Mn<sup>2+</sup>, v2.0, CH4, v2.0, CI4, DN4, CN4, SB3, SB** and Working Substrate (mixed **SB3** and **SB** reagent) with the
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skin, eyes or mucous membranes. *If contact does occur, immediately wash with large amounts of water, otherwise burns can occur.* If these reagents are spilled, dilute with water before wiping dry. *Do not allow MP LYS, which contains guanidine thiocyanate, or CH4, v2.0 and CI4, which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.*

- P. *SB and Working Substrate contain dimethylformamide, which has been reported to be toxic in high oral doses and may be harmful to the unborn child. Skin contact, inhalation of fumes and ingestion should be avoided. If skin contact occurs, wash thoroughly with soap and water and seek medical advice immediately.*
- Q. Refer to “Precautions” in other COBAS AmpliScreen package inserts, COBAS Pooling System Guide, and the *Operator’s Manuals* for the AMPLILINK and COBAS AMPLICOR Analyzer.
- R. Closely follow procedures and guidelines provided to ensure that the specimen and control preparation is performed correctly. Any deviation from the given procedures and guidelines may affect optimal assay performance.

## Reagent Preparation

### MP IC, MP (+) C, MP (–) C, MP DIL and NHP

- Warm MP IC, MP (+) C, MP (–) C, MP DIL and NHP to room temperature before use by using a 37°C incubator on laboratory bench top.

### Working Lysis Reagent

- Warm MP LYS to 25 – 37°C to dissolve precipitate (maximum 30 minutes). Mix thoroughly until the crystals are dissolved. Before use, visually verify that

crystals are dissolved and examine each bottle of **MP LYS** against a white background. Discard **MP LYS** if there is a yellow color or signs of leakage.

- Vortex **MP IC** briefly before use. Tap vial to collect the solution in the base. Pipette 100 µL **MP IC** into 1 bottle **MP LYS**. Cap the **MP LYS** bottle and vortex briefly. The pink color confirms that the **MP IC** has been added to the **MP LYS**. Discard the remaining **MP IC**.
- Store Working Lysis Reagent at room temperature. Use within 4 hours of preparation.

#### **Working Amplification Master Mix**

- Prepare Working Master Mix in a template-free area (e.g., in a dead air box).  
*Reagent preparation area must be clean and disinfected in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.*
- Pipette 100 µL HCV **Mn<sup>2+</sup>, v2.0** into 1 bottle **HCV MMX, v2.0**. Recap **HCV MMX, v2.0** bottle and mix well by inverting 10-15 times. The pink color confirms that the **HCV Mn<sup>2+</sup>, v2.0** has been added to the **HCV MMX, v2.0**. Discard the remaining **HCV Mn<sup>2+</sup>, v2.0**. Do not vortex the Working Master Mix. These reagents do not need to be at room temperature before use.
- Store at 2 – 8°C and use within 4 hours of preparation.

#### **Working Probe Suspension Detection Reagents**

- Prepare Working HCV Probe Suspension: Mix **CH PS1, v2.0** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **CH PS1, v2.0** into one **CH4, v2.0** cassette.
- Prepare Working IC Probe Suspension: Mix **CI PS1** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **CI PS1** into one **CI4** cassette.
- Both Working Probe Suspension Detection Reagents are stable for 30 days at 2 – 8°C. Working Reagents can be used for a maximum of six instrument

cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.

- Store Working Probe Suspension Detection Reagents at 2 – 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

#### **DN4 — Denaturation Reagent and CN4 Conjugate Reagent**

- Once opened, **DN4** and **CN4** are stable for 30 days at 2 – 8°C, or until the expiration date, whichever comes first. Both **DN4** and **CN4** can be used for a maximum of six instrument cycles (12 hours per cycle).
- Store **DN4** and **CN4** at 2 – 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

#### **Working Substrate Reagent**

- Working Substrate must be prepared each day by pipetting 5 mL **SB** into one **SB3** cassette. Pipette up and down at least 5 times to mix.
- Working Substrate is stable on the COBAS AMPLICOR Analyzer for a maximum of 16 hours.
- Do not expose **SB3**, **SB** or Working Substrate to metals, oxidizing agents, or direct light.

#### **Wash Buffer Reagent**

- Examine **WB** before dilution and if necessary, warm at 30 – 37°C to dissolve any precipitate. Add 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3 – 4 liters of Working Wash Buffer (1X) in the Wash Buffer Reservoir of the COBAS AMPLICOR Analyzer at all times.

- Working Wash Buffer (1X) should be stored at 2 – 25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation.

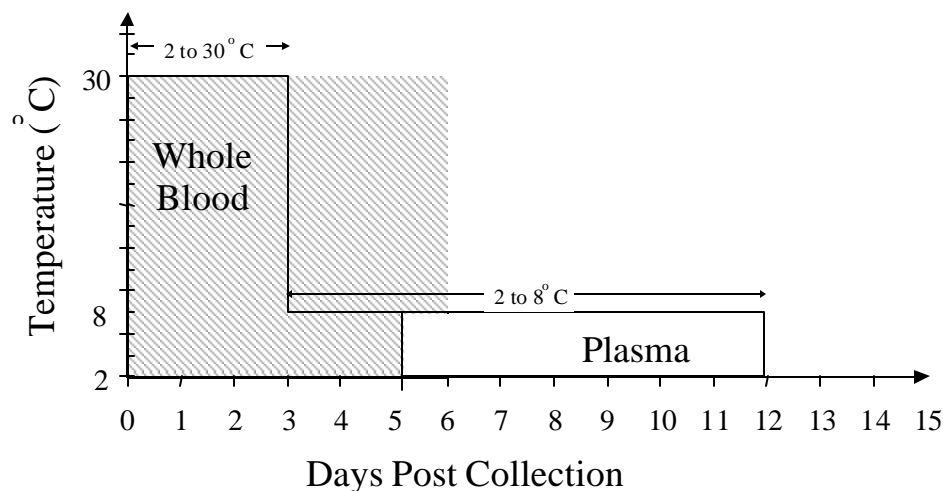
#### **70% Ethanol**

- Prepare 70% ethanol fresh daily.
- One mL 70% ethanol is needed for each specimen and control processed. For example, mix 11.7 mL 90% ethanol and 3.3 mL of distilled or deionized water for every 12 specimens and controls to be processed.

## Specimen Collection, Storage and Pooling

**Note:** *Handle all specimens as if they are potentially infectious agents.*

- A. EDTA, CPD, CPDA-1, CP2D, ACD-A and 4% Sodium Citrate may be used with the COBAS AmpliScreen HCV Test, v2.0. Follow sample tube manufacturer's instructions.
- B. Blood collected in EDTA may be stored at 2 – 30°C for up to 72 hours from time of draw, followed by an additional two days at 2 – 8°C. For storage longer than five days, remove the plasma from the red blood cells by centrifugation at 800 – 1600  $\times$  g for 20 minutes. Following removal, plasma may be stored at 2 – 8°C for an additional seven days. Alternatively, plasma may be stored at  $-18^{\circ}\text{C}$  for up to one month.



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- C. Blood collected in CPD, CPDA-1, or CP2D may be stored for up to 72 hours at 1 – 24°C. Following centrifugation of the CPD, CPDA-1, or CP2D samples at 800-1600  $\times g$  for 20 minutes, plasma may be stored at 1 – 6°C for an additional 7 days from the date the plasma was removed from the red blood cells. Plasma separated from the cells may be stored at = -18°C for up to one month.
- D. ACD-A or 4% sodium citrate anticoagulated apheresis plasma can be stored at 1 – 6°C for up to 6 hours, followed by subsequent storage at = -18°C for up to one month.
- E. Do not freeze whole blood.
- F. **Heparin has been shown to inhibit PCR. Use of heparinized specimens is not recommended.**
- G. Warm pooled or individual donor specimens to room temperature before using.
- H. Covered Archive Plates may be stored at 2 – 8°C for up to 7 days from the date the plasma was removed from the red blood cells.
- I. No adverse effect on assay performance was observed when plasma specimens were subjected to three freeze-thaw cycles.
- J. Thaw frozen specimens at room temperature before using.
- K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.<sup>14</sup>
- L. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**
- M. Specimen Pooling:

The COBAS AmpliScreen Pooling System performs barcode scanning and pooling operations that combine aliquots from 24 individual samples into a single Primary

Pool that is used for testing. The pooling algorithm requires preparation of Secondary Pools as well as individual specimens for follow-up testing in the event a Primary Pool tests positive. If less than 24 specimens are available, testing is performed using the individual specimens.

The user must validate other pooling algorithms and equipment.

## Procedural Notes

### A. Run Size

1. Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one preparation of the COBAS AmpliScreen Multiprep Negative (–) Control and one preparation of the COBAS AmpliScreen Multiprep Positive (+) Control must be included in each run (*see “Quality Control” section*).
2. The Specimen Preparation and Amplification Reagents are packaged in eight single-use bottles. The Multiprep Negative (–) and Multiprep Positive (+) Controls are packaged in single-use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.
3. The use of sterile gauze, when uncapping sample tubes may reduce the potential for cross contamination between specimens.

### B. Equipment

1. Prepare the COBAS AMPLICOR Analyzer and AMPLILINK Data Station for use according to instructions in the *Operator's Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer.
2. Prepare the Hamilton Microlab AT plus 2 System and SUNPLUS Data Station for use according to instructions in the *Operator's Manuals*.

3. Pre-cool the high-speed centrifuge and rotor to 2 – 8°C. *See* operating instructions for the high speed centrifuge for details.
4. Perform manufacturer recommended maintenance and calibration on all instruments, including pipettors, to ensure proper functioning.

### C. Reagents

1. All reagents ***except* HCV MMX, v2.0** and **HCV Mn<sup>2+</sup>, v2.0**, must be at room temperature before use. Visually examine reagents for sufficient volume before beginning the test procedure. See section “Reagent Preparation” for specific reagent storage conditions.
2. Add all reagents using a pipettor capable of delivering specified volume with  $\pm 3\%$  accuracy and a precision of  $\leq 5\%$  CV. Check pipettor functionality and calibrate as recommended by pipettor manufacturer.
3. Prepare Working Master Mix in a template-free area (e.g., in a dead air box).  
***Reagent preparation area must be clean and disinfected in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.***
4. Prepare 70% ethanol fresh each day.
5. Check expiration date of opened or Working Reagents before loading on the COBAS AMPLICOR Analyzer.
6. Check to ensure that all reagents used are of the same master lot of kit reagents.

### D. Workflow

1. To minimize the possibility of laboratory areas becoming contaminated with amplicon, the laboratory area should be separated into several distinct areas organized around Pre-Amplification and Post-Amplification. Personnel should use proper anti-contamination safeguards when moving between areas.

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2. The Pre-Amplification Area should have a template-free area for preparation of Working Master Mix and an amplicon free area for specimen and control preparation.
  3. The Post Amplification Area should have a COBAS AMPLICOR Analyzer(s) and AMPLILINK Data Station(s) with additional area for preparing Working Amplification and Detection Reagents.
  4. Pipettors and other supplies should be dedicated to a specific area. Samples, equipment and reagents should not be returned to the area where a previous step was performed.

#### **E. Temperature**

Room temperature is defined as 15° to 30°C.

#### **F. Vortexing**

Proper vortexing during sample preparation is important to ensure homogeneous mixture after additions of reagents.

#### **G. Pipetting**

1. Pooled or individual plasma specimens must be at room temperature before pipetting.
2. Use a clean pipette tip or disposable transfer pipette with each specimen or control. Use aerosol barrier or positive displacement RNase-free tips.
3. Confirm that all pipettors are correctly set to dispense the specified volumes in accordance with the specimen preparation procedures and guidelines.

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## H. Specimen Processing

1. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. Do not use snap cap tubes.
2. Avoid contaminating gloves when manipulating specimens.
3. Specimens and controls should be prepared in a laminar flow hood. ***Failure to do so may result in sample contamination.*** Specimen and control preparation area must be cleaned and disinfected in accordance with methods outlined in “Precautions” (Item A).

## I. Decontamination

Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70% ethanol.

# Instructions for Use

The Multiprep Specimen Processing Procedure is used for extracting nucleic acid from pooled specimens. The Standard Specimen Processing Procedure is used for extracting nucleic acid from individual specimens as well as for tertiary resolution.

The Multiprep and the Standard Specimen Processing Procedures are generic nucleic acid extraction procedures and can be used for the extraction HCV RNA, HIV-1 RNA, and/or HBV DNA. A single extraction is sufficient for multiple assays.

Workflow can be performed on the same day or over multiple days under the following conditions:

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## Amplification, Hybridization and Detection of Stored Processed Specimens

Amplification, hybridization and detection can occur on the same day as specimen processing or on a subsequent day. If amplification, hybridization and detection are to be done on a subsequent day, perform the Multiprep Specimen Processing Procedure described in steps B1 through B21 or the Standard Specimen Processing Procedure described in steps B22 through B38. Store the processed specimens and controls as indicated. On the subsequent day, begin with Step A (Reagent Preparation — Working Master Mix), thaw processed specimens and controls at room temperature, and continue with Step B39.

## Hybridization and Detection of Stored Denatured Amplicon

Hybridization and detection of the denatured amplicon may occur on the same day as amplification or on a subsequent day. If hybridization and detection are to be done on a subsequent day, the denatured amplicon may be left on-board the COBAS AMPLICOR Analyzer for not more than 24 hours before starting the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2 – 8°C for not more than five days before starting the hybridization and detection steps.

### A. Reagent Preparation — Working Master Mix

*Performed in: Pre-Amplification — Reagent Preparation Area (e.g., dead air box)*

- A1. Determine the appropriate number of A-ring(s) needed for specimen and control testing.
- A2. Place the A-ring(s) on the A-ring holder(s).
- A3. For each A-ring, prepare one Working Master Mix.

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- A4. Pipette 50 µL Working Master Mix into each A-tube. Discard unused Working Master Mix. Do not close the covers of the A-tubes at this time.
  - A5. Place the A-ring containing Working Master Mix in a sealable bag and seal the plastic bag. Record the assay name (HCV) and the time the Working Master Mix was prepared.
  - A6. Store the A-ring(s) containing Working Master Mix at 2 – 8°C until specimen and control preparation is completed. The A-rings with Working Master Mix must be used within 4 hours of preparation.
  - A7. Decontaminate area. *See* “Procedural Notes.”

## **B. Specimen and Control Preparation**

*Performed in Pre-Amplification — Specimen and Control Preparation Area*

### **Multiprep Specimen Processing Procedure**

- B1. Pipette 1000 µL of each pool into an appropriately labeled screw-capped tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes.
- B2. Vortex NHP briefly.
- B3. For each Negative and Positive Control pipette 1000 µL NHP into an appropriately labeled screw-capped tube. Cap the tubes.
- B4. Use a permanent marker to make an orientation mark on each tube.
- B5. Place the specimen and control tubes into the pre-cooled high-speed centrifuge with the orientation marks facing outward, so that the orientation marks will align with the pellets formed during centrifugation.
- B6. Centrifuge specimens and control tubes at 23,000 – 24,000  $\times g$  for 60 ± 4 minutes at 2 – 8°C. The pellet will form on the outer wall as indicated by the orientation

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mark. *Note: The  $60 \pm 4$  minutes begins when the centrifuge reaches  $23,000 - 24,000 \times g$ .*

- B7. Remove the tubes from the centrifuge and remove the caps. Slowly aspirate 900  $\mu$ L of the supernatant from each centrifuged tube leaving approximately 100  $\mu$ L of supernatant. Avoid contact with the pellet. Discard the supernatant and pipette tip appropriately. Use a fresh pipette tip for each tube.
- B8. Prepare a Working Lysis Reagent bottle for every batch of 12 specimens and controls to be processed.
- B9. Pipette 600  $\mu$ L Working Lysis Reagent into each specimen and control tube. Cap and vortex tubes briefly.
- B10. Prepare Controls as follows:
- a. Negative Control  
  
Vortex **MP (-) C** briefly. Tap vial to collect the solution in the base. Pipette 20  $\mu$ L **MP (-) C** to the tube labeled “MP (-) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.
  - b. Positive Control  
  
Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20  $\mu$ L **MP (+) C** to the tube labeled “MP (+) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.
- B11. Incubate all tubes for 10 to 15 minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.
- B12. Pipette 700  $\mu$ L of isopropanol into each tube. Cap the tubes and vortex briefly.
- B13. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at  $14,250 \pm 1750 \times g$  for 15 – 20 minutes at room temperature.

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- B14. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.
- B15. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.
- B16. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at  $14,250 \pm 1750 \times g$  for 5 – 10 minutes at room temperature.
- B17. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.
- B18. Using a new transfer pipette for each tube, repeat Step B17 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***
- B19. Pipette 200 µL **MP DIL** into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30 – 40 µL of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.
- B20. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***
- B21. Proceed to step **B39, Loading the A-ring**

#### **Standard Specimen Processing Procedure**

- B22. Pipette 200 µL of each specimen into an appropriately labeled screw-capped tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes.

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- B23. Vortex NHP briefly.
- B24. For each Negative and Positive Control pipette 200 µL NHP into appropriately labeled screw-capped tubes. Cap the tubes.
- B25. Use a permanent marker to make an orientation mark on each tube.
- B26. Prepare a Working Lysis Reagent bottle for every 12 specimens and controls to be processed.
- B27. Pipette 600 µL Working Lysis Reagent into each tube. Cap and vortex tubes briefly.
- B28. Prepare Controls as follows:

Negative Control

Vortex **MP (-) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (-) C** into the tube labeled “MP (-) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (+) C** into the tube labeled “MP (+) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

- B29. Incubate all tubes for 10- 15minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.
- B30. Pipette 800 µL of isopropanol into each tube. Cap the tubes and vortex briefly.
- B31. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at  $14,250 \pm 1750 \times g$  for 15-20 minutes at room temperature.
- B32. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.

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- B33. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.
- B34. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at  $14,250 \pm 1750 \times g$  for 5 – 10 minutes at room temperature.
- B35. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.
- B36. Using a new transfer pipette for each tube, repeat Step B35 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***
- B37. Pipette 200  $\mu$ L MP DIL into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30-40  $\mu$ L of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.
- B38. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***

**Loading the A-ring.**

- B39. Create an A-ring worklist record for each A-ring to identify the A-tube with the appropriate control or specimen to be pipetted.
- B40. If processed specimens and controls were stored frozen, thaw at room temperature before proceeding. Briefly vortex the processed specimens and controls.
- B41. Pipette 50  $\mu$ L of each processed specimen and control into the appropriate A-tube containing HCV Working Master Mix. Use the A-ring worklist record to ensure

the appropriate specimen or control is added to the correct A-tube position for each A-ring.

B42. Cap the A-tubes.

B43. Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area. Proceed to Part C.

***Note:** Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.*

## **C. Reverse Transcription, Amplification and Detection**

*Performed in Post-Amplification — Amplification/Detection Area*

C1. Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

- a. Wipe D-cup handler tip with a lint-free moist cloth and dry.
- b. Wipe initialization post with a lint-free moist cloth and dry.

C2. Before each run:

- a. Check waste container and empty if necessary.
- b. Check Wash Buffer Reservoir and add prepared Wash Buffer if necessary.
- c. Replace used D-cup racks.
- d. Prime the COBAS AMPLICOR Analyzer.

C3. Instrument Loading and System Operation

- a. Prepare enough of the following detection reagent cassettes to complete the workload: Working HCV Probe Suspension Reagent (**CH4, v2.0**), Working IC Probe Suspension Reagent (**CI PS1**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).

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- b. Place the **CH4, v2.0** and **CI PS1** cassettes in the test-specific reagent rack.
  - c. Place **DN4, CN4** and **SB3** cassettes in the generic reagent rack. Record on the cassette the date when each cassette was opened.
  - d. Identify the reagent racks as generic or test specific using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.
  - e. Configure the reagent racks by entering the reagent positions and lots using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.
  - f. Load the reagent racks onto the analyzer using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.
  - g. Place the D-cup rack on the D-cup platform. Two D-cups are required for each A-tube and two D-cups are required for each Working Substrate cassette to allow for blanking by the COBAS AMPLICOR Analyzer, as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
  - h. Place the A-ring into the thermal cycler segment of the COBAS AMPLICOR Analyzer and close the cover on the thermal cycler segment.
  - i. Load the A-ring into the COBAS AMPLICOR Analyzer using the Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.
  - j. Create an A-ring order, using the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Use the A-ring worklist record created for specimen processing to assist in entering the A-ring order.
  - k. Repeat steps h. through j. above to load a second A-ring on the COBAS AMPLICOR Analyzer.

- l. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for AMPLILINK software.
- m. Wait for the COBAS AMPLICOR Analyzer to indicate that the load check has passed.

**Note:** *The required quantity of each detection reagent is automatically calculated by the COBAS AMPLICOR Analyzer during the Load Check to determine if sufficient reagents are available for the requested tests.*

- n. The COBAS AMPLICOR Analyzer automatically performs reverse transcription, amplification and detection. Results are expressed as absorbance values at 660 nm and as positive or negative.
- o. For each run, print the AMPLILINK A-ring Results Report and the Run Log and retain these along with the A-ring worklist. Compare the A-ring worklist record with the AMPLILINK A-ring Results Report and verify that the A-ring ID, instrument serial number, and specimen IDs are identical. Reconcile the Run Log with the A-ring worklist to account for all A-ring IDs associated with each run. If there are discrepancies, perform follow-up investigation.

## Quality Control Procedures

1. At least one Multiprep (-) Control and one Multiprep (+) Control must be processed with each A-ring.
  - a. Negative Control

The absorbance for the **MP (-) C** should be less than 0.1 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 for the Negative Control to be valid. If the absorbance value for the **MP (-) C** is greater than or equal to 0.1 and/or its associated **MP IC** is less than 0.2, the entire run is invalid, and the entire test procedure (sample and control preparation, amplification and detection) must be repeated.

b. Positive Control

The absorbance for the **MP (+) C** should be greater than or equal to 1.0 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 at 660 nm for the Positive Control to be valid. If the absorbance value for the **MP (+) C** is less than 1.0 and/or its associated **MP IC** is less than 0.2, the entire run is invalid, and the entire test procedure (specimen and control preparation, amplification and detection) must be repeated.

**Summary of Control Acceptance Criteria**

	HCV Result		IC Result	
	A <sub>660</sub>	Comment	A <sub>660</sub>	Comment
Negative Control	< 0.1	Negative	≥ 0.2	Valid
Positive Control	≥ 1.0	Positive	≥ 0.2	Valid

- Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

3. External Control

If an External Control (i.e., an additional run control other than the Multiprep (+) Control or Multiprep (-) Control) is required by the laboratory, the External Control should meet regulatory requirements for such controls. The absorbance of the HCV External Control should be equal to or greater than 0.2 at 660 nm, irrespective of the MP IC absorbance. If the absorbance of the HCV External Control does not meet the above criterion, the negative results for specimens in the associated run may be invalidated. However, positive results for specimens in such a run should not be invalidated solely on the basis of the results obtained for an External Control; those positive results should remain the test of record. The laboratory should follow its established Standard Operating Procedure for the appropriate action.

## Interpretation of Results

1. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

2. Specimen Results

Two absorbance values are obtained for each specimen: one for the HCV target and one for the internal control (**MP IC**). For a sample with an absorbance less than 0.2, the **MP IC** absorbance for that specimen must be greater than or equal to 0.2 at 660 nm for a valid negative specimen test result. If the absorbance for the HCV target is greater than or equal to 0.2, the **MP IC** result is disregarded and the test result is valid and positive.

3. For a valid run, results are interpreted as follows:

HCV Result		IC Result		Interpretation
A <sub>660</sub>	Comment	A <sub>660</sub>	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HCV RNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HCV RNA.

### Invalid Test Runs

When invalid Positive or Negative Control results are obtained on an A-ring, that run is invalid. Repeat the entire test procedure for the associated specimens (including specimen and control preparation, amplification and detection) in the run by processing another aliquot of the original plasma specimens.

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With the exception of instrument failures subsequent to denaturation of amplicon, an instrument failure during a test run, as indicated by system error messages, also constitutes an invalid test run. In such instances, repeat the test procedure for the associated controls and specimens (amplification and detection) in the run by processing another aliquot of the processed specimen.

For instrument failures subsequent to successful denaturation of amplicon, it is not necessary to repeat the entire test procedure for the associated specimens. In such instances, the denatured amplicon may be redetected by the COBAS AMPLICOR Analyzer. The denatured amplicon may be left on the COBAS AMPLICOR Analyzer for not more than 24 hours before continuing with the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2 – 8°C for not more than five days before continuing with the hybridization and detection steps.

#### **Invalid Specimen Results**

For specimen(s) that are invalid, perform repeat testing in single on the remaining replicate tube(s). The test result for the pool or individual donor specimen is based only on the repeat valid test result. If the last available replicate of a pooled specimen gives an invalid result, each individual donor specimen in that pool should be tested. If an individual donor specimen gives an invalid result, the test result for that individual donor specimen should be considered invalid for HCV RNA.

#### **Results of Pooled Donor Specimens**

Testing of pooled samples for the COBAS AmpliScreen HCV Test, v2.0 requires a single level of testing for Primary Pools that are negative for HCV RNA and three levels of testing (Primary Pool, Secondary Pool and tertiary resolution) for Primary Pools that are positive for HCV RNA.

#### **Negative Primary Pools**

When the Primary Pool is negative, report the results for all associated individual donor specimens in that Primary Pool as “HCV RNA Negative”.

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### **Positive Primary Pools — Secondary Pool Testing**

When the Primary Pool is positive, prepare four Secondary Pools containing the associated donor specimens. The Secondary Pools must be processed using the Multiprep Specimen Processing Procedure.

- If one or more of the Secondary Pools tests positive, report the results for the donor specimens in the negative Secondary Pools as “HCV RNA Negative”. For positive Secondary Pools, proceed to the section entitled “***Positive Primary Pool, Positive Secondary Pools — Tertiary Resolution Testing.***”
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as “HCV RNA Negative.”
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool.

### **Positive Primary Pool, Positive Secondary Pools — Tertiary Resolution Testing**

For a positive Secondary Pool, test each of the individual donor specimens in that Secondary Pool. The individual donor specimens must be processed using the Standard Specimen Processing procedure.

- If one or more of the individual donor specimens is positive, the positive donor specimen(s) is (are) reported as “HCV RNA Positive” and the remaining negative donor specimens associated with the positive Secondary Pool are reported as “HCV RNA Negative.”
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as “HCV RNA Negative.”
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary and Secondary Pools.

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### **Results of Individual Donor Samples**

If an individual donor specimen is positive, the positive donor specimen is reported as “HCV RNA Positive.”

If an individual donor specimen is negative, the negative donor specimen is reported as “HCV RNA Negative.”

## **Procedural Limitations**

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, COBAS AMPLICOR Analyzer, the Hamilton Microlab AT plus 2 Pipettor for the automated preparation of plasma pools.
2. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AmpliScreen HCV Test, v2.0.***
3. Reliable results are dependent on adequate specimen collection and proper transport procedures.
4. Detection of HCV RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
5. Only the Hamilton Microlab AT plus 2 Pipettor has been validated for use with the COBAS AmpliScreen HCV Test, v2.0 for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the User Manual for the Hamilton Microlab AT plus 2 Pipettor.

## Performance Characteristics

### Reproducibility

The reproducibility of the Test was established by testing two six-member EDTA plasma panels with known concentrations of HCV. Panel One was tested using the Multiprep Specimen Processing Procedure contained one HCV-negative sample and HCV-positive samples with HCV RNA concentrations of 10, 25, 50, and 50,000 IU/mL. Panel Two was tested using the Standard Specimen Processing Procedure contained one HCV-negative sample and HCV-positive samples with concentrations of 25, 50, 100 and 50,000 IU/mL.

Testing was performed at three sites with two operators at each site using three COBAS AmpliScreen HCV Test, v2.0 kit lots. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (Multiprep and Standard).

The reproducibility study for the COBAS AmpliScreen HCV Test, version 2.0 demonstrated consistency by lot and site for both the Multiprep and Standard Specimen Processing Procedures as seen in Table 1 and 2 below:

**Table 1: Reproducibility Results— Multiprep Specimen Processing Procedure**

Results By Lot (Number Positive/Number Tested)					
	Negative	10 IU/mL	25 IU/mL	50 IU/mL	50,000 IU/mL
Lot #1	0/89	72/89	164/177	88/90	90/90
(%)	(0%)	(81%)	(93%)	(98%)	(100%)
Lot #2	0/90	59/90	168/180	88/89	90/90
(%)	(0%)	(66%)	(93%)	(99%)	(100%)
Lot #3	0/90	59/90	170/179	88/89	90/90
(%)	(0%)	(66%)	(95%)	(99%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/90	66/89	166/178	88/89	90/90
(%)	(0%)	(74%)	(93%)	(99%)	(100%)
Site #2	0/89	65/90	170/179	90/90	90/90
(%)	(0%)	(72%)	(95%)	(100%)	(100%)
Site #3	0/90	59/90	166/179	86/89	90/90
(%)	(0%)	(66%)	(93%)	(97%)	(100%)

**Table 2: Reproducibility Results— Standard Specimen Processing Procedure**

Results By Lot (Number Positive/Number Tested)					
	Negative	25 IU/mL	50 IU/mL	100 IU/mL	50,000 IU/mL
Lot #1	0/90	56/89	166/180	89/90	90/90
(%)	(0%)	(63%)	(92%)	(99%)	(100%)
Lot #2	0/90	66/89	165/179	89/90	90/90
(%)	(0%)	(74%)	(92%)	(99%)	(100%)
Lot #3	3/87	68/90	167/179	89/90	90/90
(%)	(3%)	(76%)	(93%)	(99%)	(100%)
Results By Site (Number Positive/Number Tested)					
Site #1	0/87	61/89	162/179	85/87	90/90
(%)	(0%)	(69%)	(91%)	(98%)	(100%)
Site #2	1/90	72/90	169/179	88/90	90/90
(%)	(1%)	(80%)	(94%)	(98%)	(100%)
Site #3	2/90	57/89	167/180	88/90	90/90
(%)	(2%)	(64%)	(93%)	(98%)	(100%)

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## Analytical Sensitivity — Dilutional Panels

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was determined by testing 10 HCV seropositive clinical specimens. The titer of each specimen was quantitated with a commercially available assay using a secondary standard calibrated against the WHO International Standard. These specimens were diluted in normal human plasma to 150, 50, 16.7 and 5.6 HCV RNA IU/mL for the Multiprep Specimen Processing Procedure and 300, 100, 33.3 and 11.1 IU/mL for the Standard Specimen Processing Procedure. The COBAS AmpliScreen HCV Test, v2.0 detected 16.7 HCV RNA IU/mL at a frequency greater than 90% with a lower 95% confidence limit of 86.4% using the Multiprep Specimen Processing Procedure. The assay detected 33.3 HCV RNA IU/mL at a frequency greater than 84% with a lower 95% confidence limit of 79.7% using the Standard Specimen Processing Procedure. The data are presented in Tables 3 and 4.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 21.0 IU/mL, with lower and upper 95% confidence limits of 17.1 IU/mL and 27.8 IU/mL, respectively. The LOD of 21.0 IU/mL corresponds to approximately 57 copies/mL.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 54.1 IU/mL, with lower and upper 95% confidence limits of 44.1 IU/mL and 71.7 IU/mL, respectively. The LOD of 54.1 IU/mL corresponds to approximately 146 copies/mL.

**Table 3: Multiprep Procedure Testing Summary for All Clinical Samples  
Combined Input Values with 95% One-tailed Lower Confidence Limit**

Multiprep Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
150	219	219	100.0%	98.6%
50	220	220	100.0%	98.6%
16.7	197	218	90.3%	86.4%
5.6	30	44	68.1%	54.8%

**Table 4: Standard Procedure Testing Summary for All Clinical Samples  
Combined Input Values with 95% One-tailed Lower Confidence Limit**

Standard Sample Processing Procedure				
HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – One-Tailed
300	220	220	100.0%	98.6%
100	220	220	100.0%	98.6%
33.3	183	217	84.3%	79.7%
11.1	54	87	62.1%	52.7%

## Analytical Sensitivity — WHO HCV International Standard

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was also determined using the WHO HCV International Standard (96/790). The WHO HCV International Standard was serially diluted in HCV-negative plasma to final concentrations of 200, 100, 50, 25, 15, and 10 IU/mL. Each dilution was tested with two lots of the COBAS AmpliScreen HCV Test, v2.0 using both the Multiprep and Standard Specimen Processing Procedures.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% LOD of

28.8 IU/mL, with lower and upper 95% confidence limits of 20.5 IU/mL and 85.8 IU/mL, respectively.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 41.9 IU/mL, with lower and upper 95% confidence limits of 28.0 IU/mL and 111.8 IU/mL, respectively.

Tables 5 and 6 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

**Table 5: Serial Dilution Testing Summary for Multiprep Method  
Combined Input Values with Lower 95% Confidence Limit (One-Sided)**

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	132	132	100.00%	97.76%
100	132	132	100.00%	97.76%
50	130	132	98.48%	95.31%
25	128	132	96.97%	93.20%
15	95	132	71.97%	64.83%
10	92	132	69.70%	62.45%

**Table 6: Serial Dilution Testing Summary for Standard Method  
Combined Input Values with Lower 95% Confidence Limit (One-Sided)**

HCV RNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-sided)
200	131	131	100.00%	97.74%
100	129	132	97.73%	94.23%
50	132	132	100.00%	97.76%
25	115	132	87.12%	81.31%
15	93	131	70.99%	63.77%
10	84	132	63.64%	56.19%

## Analytical Sensitivity — CBER HCV Panel

The FDA CBER HCV Panel Members # 1-10 were processed using the Multiprep and Standard Sample Processing Procedures. Both specimen processing methods detected HCV RNA at 50 copies/mL. The Multiprep Sample Processing Procedure detected 100% of all positive members ranging from 10 - 100,000 copies/mL. The Standard Sample Processing Procedure detected 100% of all positive members ranging from 50 to 100,000 copies/mL. Both negative members of the panel were negative by both methods. The data are shown in Table 7.

**Table 7: CBER HCV RNA Panel Results**

CBER HCV RNA Panel (Copies/mL)	CBER HCV Panel Member Test Results (Percent Positive)									
	1 (1000)	2 (Neg)	3 (100,000)	4 (10,000)	5 (Neg)	6 (500)	7 (200)	8 (50)	9 (10)	10 (5)
Multiprep Method	100%	0%	100%	100%	0%	100%	100%	100%	100%	67%
Standard Prep Method	100%	0%	100%	100%	0%	100%	100%	100%	67%	0%

## Genotype Detectability

Twenty individual plasma specimens representing Genotypes 1 and 4, sixteen plasma specimens of Genotype 2, nineteen plasma specimens of Genotype 3, and two plasma specimens each of Genotypes 5 and 6 were tested. With the exception of one sample (Genotype 2a/2c), which was below the limit of quantitation by a quantitative assay, each specimen was diluted to approximately 200 IU/mL of HCV RNA in pooled negative human plasma. Diluted samples were processed using both the Multiprep and Standard Sample Processing Procedures. The COBAS AmpliScreen HCV Test, v2.0 detected all Genotypes at 200 IU/mL except the one sample that was not quantifiable. This sample (Genotype 2a/2c) was detected using the Multiprep Specimen Processing Procedure, but was negative when tested using the Standard Specimen Processing Procedure. This result

is consistent with HCV RNA levels below the detection limit of the assay. Data are provided in Table 8.

**Table 8: HCV Genotype Samples Tested**

HCV Genotype/Subtype	Quantity	Reactive/Total (Multiprep)	Reactive/Total (Standard Prep)
1	8	8/8	8/8
1a	3	3/3	3/3
1b	9	9/9	9/9
2	1	1/1	1/1
2a	2	2/2	2/2
2b	10	10/10	10/10
2a/2c	3	3/3	2/3*
3a	12	12/12	12/12
3a	6	6/6	6/6
3e	1	1/1	1/1
4	1	1/1	1/1
4	11	11/11	11/11
4a	2	2/2	2/2
4c	3	3/3	3/3
4c/4d	2	2/2	2/2
4h	1	1/1	1/1
5a	2	2/2	2/2
6a	2	2/2	2/2

\* One sample contained HCV RNA at a level below the Limit of Quantitation of a quantitative assay. Sample was tested undiluted.

## Seroconversion Panels

Nine anti-HCV seroconversion panels were tested using both the Multiprep and the Standard Specimen Processing Procedures. Each specimen in each panel was tested by the Ortho HCV, version 3.0 ELISA Test system and all samples with reactive EIA results were also tested by Chiron RIBA HCV 3.0 SIA. The HCV RNA test results were then compared to the EIA test results for each specimen to determine if HCV RNA testing detected the presence of HCV infection prior to seroconversion.

The COBAS AmpliScreen HCV Test, v2.0 detected HCV infection an average of 32 days before seroconversion for the nine seroconversion panels. The data are summarized in Table 9.

**Table 9: HCV Seroconversion Study**

Panel	Day Positive Ortho 3.0 EIA and Chiron RIBA 3.0	Day Positive AmpliScreen v2.0	Difference AmpliScreen vs EIA
6212	14	0	14
6224	19	0	19
6215	20	0	20
9047	28	0	28
9045	41	0	41
6225	78	39	39
6213	43	11	32
6222	40	17	23
6227	74	0*	74*
Mean Days Earlier Detection			32

\* Specimen was RNA positive on Day 0 but negative on Days 22 and 24. Day 74 specimen was RNA positive again

## Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the COBAS AmpliScreen HCV Test, v2.0 was evaluated by testing a panel of microorganisms and other disease states, including 23 viral isolates, two bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS AmpliScreen HCV Test, v2.0. Table 10 summarizes the microorganisms studied.

**Table 10: Analytical Specificity — Microorganisms Tested**

Adenovirus type 2	Epstein Barr Virus	HIV-1 Subtype D
Adenovirus type 3	Hepatitis A Virus	HIV-2
Adenovirus type 7	Hepatitis B Virus (n=3)	HTLV-I
Autoimmune samples	Herpes Simplex type 1	HTLV-II
<i>Candida albicans</i>	Herpes Simplex type 2	Human Herpes Virus 6
<i>Chlamydia trachomatis</i>	HIV-1 Subtype A	Human Herpes Virus 7
Coxsackievirus B1	HIV-1 Subtype B	<i>Staphylococcus epidermidis</i>
Cytomegalovirus	HIV-1 Subtype C	Varicella-Zoster
Echovirus 1		

Up to ten individual patient plasma specimens from each of the following disease categories were spiked with low levels of HCV-positive plasma (within 2-3X the 95% LOD): HIV-1, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*. No false negative test results were observed.

## Analytical Specificity — Non-HCV Hepatitis Samples

Twenty-five HAV- and 25 HBV-positive specimens (all HCV-negative) were tested for cross reactivity with the COBAS AmpliScreen HCV Test, v2.0 by using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be negative. No false positive test results were observed.

These samples were also spiked with low levels of HCV-positive plasma and tested using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be positive. No false negative test results were observed.

## **Potentially Interfering Substances**

### **Endogenous Interfering Substances**

HCV-spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HCV Test, v2.0, using either the Standard or Multiprep Specimen Processing Procedure.

### **Exogenous Interfering Substances**

HCV-spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity using either the Standard or Multiprep Specimen Processing Procedure.

## **Clinical Performance**

### **Chronic HCV Population**

Fifty-eight specimens were obtained from patients with a diagnosis of chronic HCV disease. All specimens were confirmed to be serologically positive by a licensed anti-HCV EIA followed by RIBA 3.0. The specimens were tested undiluted using the Standard Specimen Processing procedure and diluted 1:24 using the Multiprep Specimen Processing procedure. All specimens were positive in the COBAS AmpliScreen HCV Test, v2.0 by both specimen processing procedures.

### **High Risk Population**

Specimens were prospectively collected from a patient population being evaluated at hematology clinics for biochemical, clinical and/or histological evidence of liver disease and/or evidence of HCV infection. Specimens were tested in a blinded fashion with COBAS AmpliScreen HCV Test, v2.0 using the Standard Specimen Processing Procedure.

Fifty-seven of 62 total specimens were positive for HCV RNA. Four specimens negative for HCV RNA were also negative for HCV antibody by both a licensed screening EIA and confirmatory assay and were excluded from the analysis. The COBAS AmpliScreen HCV Test, v2.0 detected 57 out of 58 HCV antibody-positive specimens.

### **Pool Reactivity in Volunteer Blood Donors**

A random selection of 8,240 pools revealed that 117 Primary Pools were reactive for an initial reactive rate of 1.42%. There were 106/117 (90.6%) positive pools that were concordant with confirmed positive serology status. None of these pools were identified as having a window period case. A total of 11 pools were found positive but were not

confirmed positive by serology or by subsequent testing of individual donations by the COBAS AmpliScreen Test, v2.0. Results are summarized in Table 11.

**Table 11: Pool Reactivity in Volunteer Blood Donors**

Category	Pools	Percentage
Pools Tested	8,240	100
Non-Reactive Pools	8,123	98.58
Initially reactive pools	117	1.42
Initial pools with concordant serology	106	1.28
Positive pools due to window case	0	0
Initial Pools with negative serology and negative individual donation AmpliScreen Testing (false positive)	11	0.13

A random selection of approximately 250,000 specimens was selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS AmpliScreen HCV Test, v2.0. Using the antibody results, the HCV status of each specimen was determined. HCV status-negative included either: 1) anti-HCV EIA negative, regardless of other results (unless the subject was enrolled in the follow-up study and had test results that changed this assessment); or 2) anti-HCV EIA positive and RIBA negative.

HCV status-positive included either: 1) anti-HCV EIA repeat reactive and RIBA positive; or 2) anti-HCV EIA repeat reactive or HCV RNA positive upon follow-up. HCV status-unknown included anti-HCV EIA repeat reactive with RIBA indeterminate or unknown.

There were 247,998 specimens that were determined to be HCV status-negative. Of these, 247,990 were also HCV RNA-negative. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 247,990/247,998 or 99.997% with 95% confidence limits of 99.99% to 100.00%. The negative predictive value obtained by summing all the cases determined to have HCV status negative among the 247,990 COBAS HCV tested donations is estimated in this study to be 99.95% with exact 95% confidence limits (99.94% , 99.96%).

There were 243 specimens that were determined to be status-positive. Of these, 203 were also HCV RNA-positive. The positive predictive value obtained by finding the percentage of specimens detected to be HCV status positive among 203 COBAS positive donations is estimated to be 94.42% with exact 95% confidence limits (90.45%, 97.08%). All 243 samples in this population were included in the analysis, irrespective of HCV RNA titers. These data are consistent with previous reports that about 20% of HCV seropositive samples will have undetectable HCV RNA.

## **Detection of Window Period Cases**

From April 8, 1999 to December 31, 2000, approximately 7 million donations were tested. During this period there were 20 confirmed window period cases detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive with the COBAS AmpliScreen HCV Test, v2.0 but non-reactive by EIA for anti-HCV, and a follow-up specimen was shown to be anti-HCV EIA repeat reactive using the Abbott HCV EIA 2.0 assay and/or the Ortho HCV Version 3.0 ELISA test system and/or HCV RNA positive. The detection rate of such window period cases was 0.00029% (1 in 350,000) with a 95% confidence interval of 0.00017% to 0.00041%. In addition, four subjects with negative serology and no follow-up specimens were presumed to be window period cases, as a specimen from the plasma bag for each confirmed the index HCV RNA positive result. If these four subjects are included, the detection rate of window period cases is 0.00034% (1 in 292,000) with a 95% confidence interval of 0.00021% to 0.00049%.

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## Single Donation Testing Performance

A total of 2,515 blood donor specimens were tested individually in the COBAS AmpliScreen HCV Test, v2.0 clinical trial. Of the 2515 specimens, five were classified as HCV seropositive and were removed from the calculation of specificity. Of the 2,510 specimens tested, 2,508 were HCV RNA negative and two were HCV RNA positive. No follow-up was conducted on these two donors and they were presumed to be false positive. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 99.92% (2,508/2,510) with a 95% confidence interval of 99.71% to 99.99%.

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